Rationale and design for TIME: A phase II, randomized, double-blind, placebo-controlled pilot trial evaluating the safety and timing of administration of bone marrow mononuclear cells after acute myocardial infarction

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Several previous studies have demonstrated that administration of autologous bone marrow–derived mononuclear cells (BMMNCs) improves cardiac function in patients after acute myocardial infarction (AMI). However, optimum timing of administration has not been investigated in a clinical trial. The Cardiovascular Cell Therapy Research Network was developed and funded by the National Heart, Lung, and Blood Institute to address important questions such as timing of cell delivery and to accelerate research in the use of cell-based therapies. The TIME trial is a randomized, phase II, double-blind, placebo-controlled clinical trial. The 5 member clinical sites of the Cardiovascular Cell Therapy Research Network will enroll 120 eligible patients with moderate-to-large anterior AMIs who have undergone successful percutaneous coronary intervention of the left anterior descending coronary artery and have a left ventricular (LV) ejection fraction \( \leq 45\% \) by echocardiography. Participants will have bone marrow aspirations and intracoronary infusions of 150 × 10^6 BMMNCs or placebo on day 3 or day 7 post-AMI. Objectives of this study are (1) to evaluate effects of BMMNCs on regional and global LV function compared to placebo therapy in patients with acute AMI as assessed by cardiac magnetic resonance imaging at 6 months and (2) to assess whether effects of BMMNC infusion on global and regional LV function and safety are influenced by the time of administration. This study will provide further insight into the clinical feasibility and appropriate timing of autologous BMMNC therapy in high-risk patients after AMI and percutaneous coronary intervention. (Am Heart J 2009;158:356-63.)

The development of new strategies to improve left ventricular (LV) function after acute myocardial infarction (AMI) has been a prominent goal for cardiovascular investigation. Although endogenous repair mechanisms appear limited in humans, studies in animal models have demonstrated that myocardial function can be significantly improved with bone marrow–derived stem cells after experimental AMI. 1-4 Although data supporting significant myocardial regeneration in these preclinical studies have not been uniform,5,6 it has led to a number of clinical trials testing the strategy that delivery of autologous bone marrow–derived mononuclear cells (BMMNCs) into the infarct region after AMI may improve LV function.7-10

Meta-analyses of AMI stem cell trials11,12 have confirmed that BMMNC administration appears safe over several
under the aegis of the NHLBI. Each clinical center and the Therapies Data Safety and Monitoring Board (DSMB) network protocols that are also reviewed by an independent institutional review board approvals and oversight. By recruiting from multiple centers, the Network accelerates the speed with which its studies can be completed, increases the generalizability of study findings, and improves the dissemination of its findings to influence public health.

**Objectives and design**

The objectives of this study are (1) to evaluate the effects of stem cell therapy on cardiovascular function when compared to control therapy in patients with an acute anterior myocardial infarction (AMI) as assessed by cardiac magnetic resonance imaging (cMRI) and (2) to assess whether the effect of this BMMNC infusion on regional and global LV function is influenced by whether it is given at 3 days versus 7 days post-AMI. The primary outcome will be change in regional function (ie, segmental shortening, thickening, and radial displacement) and global LV function (ie, LVEF) at 6 months compared to baseline as measured by cMRI. Patients will be followed up for 2 years to evaluate the effect of therapy on the clinical events of death, repeat revascularization, MI, and hospitalization for congestive heart failure (CHF). A secondary objective will be to examine the effects of cell phenotype on therapeutic efficacy through ancillary studies at a Biorepository Core.

**Hypotheses and study power**

The primary hypotheses of the TIME study are that, as compared with placebo therapy, (1) administration of cell therapy will improve global and regional LV function and (2) this improvement will depend on the timing of cell delivery. The secondary hypotheses are that, in comparison with control therapy, (1) administration of cell therapy will result in smaller end-diastolic and end-systolic volumes (2) and a lower incidence of the composite adverse events of death, infarction, repeat revascularization, and hospitalization for CHF.

**Enrollment and study population**

This study is a randomized, double-blind, placebo-controlled clinical study of autologous BMMNC administration to 120 patients after AMI. Patients will be randomized to a 2:1 treatment-versus-placebo therapy ratio at each of the 2 time points. Patients enrolled in this study will be recruited from each of the 5 sites participating in the CCTRN. Enrollment will be limited to patients with moderate-to-large anterior infarctions with no prior history of coronary artery bypass grafting (CABG) and whose LVEF after percutaneous coronary intervention (PCI) is ≤45%. Because of the experimental nature of cell therapy and limited long-term safety data, only patients who are at increased risk of death or major
Table I. Inclusion and exclusion criteria for TIME

**Inclusion criteria**

1) Patients at least 21 years of age
2) Patients with first acute anterior MI with successful primary PCI in the left anterior descending artery at least 2.5 mm in diameter within 24 hours of onset of symptoms
3) Hemodynamic stability as defined as no requirement for IABP, inotropic, or blood pressure supporting medications
4) Ejection fraction after reperfusion with PCI ≤45% as assessed by echocardiography
5) Consent to protocol and agree to comply with all follow-up visits and studies
6) Women of child-bearing potential willing to use an active form of birth control

**Exclusion criteria**

1) History of sustained ventricular arrhythmias not related to their AMI (evidenced by previous Holter monitoring and/or medication history for sustained ventricular arrhythmias in patient’s medication chart)
2) Requires CABG or PCI due to the presence of residual coronary stenosis >70% luminal obstruction in the non–infarct-related vessel (in addition, PCI of nonculprit vessels may be performed before enrollment)
3) History of any malignancy within the past 5 years excluding nonmelanoma skin cancer or cervical cancer in situ
4) History of chronic anemia (hemoglobin <9.0 mg/dL)
5) History of thrombocytosis (platelets >120,000 or known history of thrombocytopenia
7) Known history of elevated INR (PT) or PTT
8) Life expectancy <1 y
9) History of untreated alcohol or drug abuse
10) Currently enrolled in another investigational drug or device trial
11) Previous CABG
12) Previous MI or history of nonischemic cardiomyopathy resulting in LV dysfunction (LVEF <55%)
13) History of stroke or transient ischemic attack within the past 6 m
14) History of severe valvular heart disease (aortic valve area <1.0 cm² or >3 + mitral regurgitation)
15) Pregnancy or breast-feeding
16) Subjects with a known history of HIV, hepatitis B or C infection, or TB-positive therapy
17) Patients with active inflammatory or autoimmune disease on chronic immunosuppressive therapy
18) Contraindications to cMRI
19) Previous radiation to the pelvis with white blood cell count and platelet counts below hospital specific normal values
20) Women of child-bearing potential not willing to practice an active form of birth control
21) Hepatic dysfunction as defined by aspartate aminotransferase or alanine aminotransferase ≥3 times the upper limit of normal, or total bilirubin ≥2 times upper limit of normal with aspartate aminotransferase or alanine aminotransferase ≥2 times upper limit of normal
22) Chronic renal insufficiency as defined by a creatinine ≥2.0 mg/dL or requires chronic dialysis
23) The revascularized vessel is not patent at the time cell administration is to be attempted
24) Patient with 2 or more of the following criteria will be excluded from the trial, unless the LVEF is < 30%:
   1. Onset of symptoms to treatment PCI <2 h
   2. Peak CK <1500 IU/mL
   3. Absence of q-wave on day 1 EKG (poststenting)
   4. Age <45 y

Table II. Final product release criteria testing

<table>
<thead>
<tr>
<th>Assay</th>
<th>Test method</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Product release specification</td>
<td>Rapid Sterility Endotoxin</td>
<td>No organisms</td>
</tr>
<tr>
<td></td>
<td>Viability Trypan Blue EndoSafe PTS</td>
<td>≥70% ≤5 EU/kg</td>
</tr>
<tr>
<td></td>
<td>TNC Manual or automated</td>
<td>Not more than 150 x 10⁶</td>
</tr>
<tr>
<td>2. Postproduction monitoring</td>
<td>Immunophenotyping Flow cytometry</td>
<td>Report</td>
</tr>
<tr>
<td></td>
<td>CFU Per site SOP</td>
<td>Report</td>
</tr>
<tr>
<td></td>
<td>Sterility 14-D culture</td>
<td>No growth</td>
</tr>
</tbody>
</table>

TNC, Total nucleated cells; SOP, standard operating procedure.

adverse events, including recurrent MI or development of CHF, will be eligible for cell therapy administration. All prospective patients will be screened and enrolled in the trial after meeting inclusion/exclusion criteria (Table I) and signing both the informed consent and Health Insurance Portability and Accountability Act (HIPAA) forms.

Randomization to the timing of administration

The clinical center will electronically transmit eligibility criteria to the DCC, at which time a computer-generated scheme will randomly allocate eligible patients (1:1) to an intervention time group (3 or 7 days post-PCI, with day zero defined as the day of incident PCI). This randomization will be not be blinded, and participants will be stratified by clinical center. Patients randomized to day 3 therapy must receive cMRI, bone marrow aspirations, cell processing, and therapy infusions on the third day post-PCI. Patients randomized to day 7 therapy receive a day 3 cMRI and have bone marrow aspirations, a repeat cMRI, cell processing, and cell therapy infusions on day 7 post-PCI. An adjustment of 1 day is permitted should the assigned day of administration fall on a weekend or holiday.

Bone marrow aspiration and cell processing

On the morning of the study product administration, patients will undergo bone marrow aspiration in accordance with standard operating procedures developed by each CCTRN site. Approximately 80 to 90 mL of bone marrow are aspirated under appropriate anesthesia from the iliac crest using standard techniques and transported to the institution’s cell-processing laboratory. Each site will use the Sepax System (Biosafe, Eysins, Switzerland) for BMMNC isolation. This approved closed system device for cord blood processing produces a faster isolation and more uniform cellular product. This will be the first cardiovascular stem cell trial to use this method of cell isolation.
The cells will be harvested and washed 3 times in heparinized phosphate-buffered saline before resuspen-
sion in phosphate-buffered saline supplemented with 5%
human albumin. The composition of CD34+, CD45+, and
CD133+ cells will be determined by fluorescent activated
cell sorting analysis. Viability of the cells will be
determined by Trypan Blue exclusion; and ≥70%
viability will be required before transplantation. A 14-
day sterility culture, colony forming unit (CFU) Assay,
and endotoxin analysis will be performed on the final
product. Because 14-day sterility testing will not be
available before the product's infusion, a negative Gram
stain will be required before the product is released
(Table II). Products are labeled and tracked with
adhesive labels containing the patient's name and
hospital identification number.

Approximately 150 to 200 million nucleated cells can
be routinely harvested with this volume of bone
marrow. Target dose will be determined using a
hematology analyzer. Because the specific cell type(s)
responsible for the previously observed biologic effect in
the infarct zone has not been identified, unfraccionated
BMMNC will be used. Characteristics of the specific
population of cells administered in this study will be
investigated by the CCTRN Biorepository and correlated
with major outcomes to help address this question.
Although autologous cells will be used in this protocol,
standard tests for infectious diseases, including HIV and
HCV (by nucleic acid testing), anti-HIV I/II, anti-HTLV I/
II, anti-HBc antibody (Ab), HBsAg, anti-HCV, and Trepon-
ema pallidum (by serology) will be performed. Cells
testing positive for infectious disease markers will be
labeled as infectious and quarantined while in the
Clinical Cell Therapy Laboratory facilities. If any test is
positive, the patient will be notified of the result via the
medical director, principal investigator, and patient
physician within 48 hours for appropriate clinical action.

After the product has passed the prospectively described
release criteria, an unblinded cell processor will enter the
data into a Web-based computer application that transmits
the data to the DCC. The patient will then be assigned to
cell therapy or placebo intervention. Patients randomized
to cell therapy will receive 150 million cells. Any patient
randomized to the cell therapy arm whose BM aspiration
produces less than the target dose will receive all the
available cells harvested. Cells in excess of 150 million will
be sent to the CCTRN Biorepository core laboratories at
the University of Florida and the University of Minnesota
for cell characterization. Patients who are randomized to
receive placebo therapy will receive 5% human serum
albumin in an identical volume of saline with a 100 μL
blood matching the appearance of an active cell
preparation and thereby blinding the identity of the
infusate being delivered. Their cells will be sent to the
Biorepository core laboratory.

**Infusion of cellular product**

The final cellular product or placebo will be infused
within 6 to 12 hours after bone marrow aspiration (total
volume, 30 mL). The patients are heparinized to an
activated clotting time (ACT) ≥200 seconds, and the
infusate is delivered via an over-the-wire percutaneous
transluminal coronary angioplasty (PTCA) catheter in 6
aliquots (5 mL), each delivered over 2 minutes of balloon
inflation within the previously placed stent at low
pressure (3-4 atm). Two minutes of reperfusion will
occur after each cycle of cell infusion. Patients are
routinely discharged the following day, and they are
advised to take aspirin and 75 mg of clopidogrel for 24
months, as well as the usual post-AMI care medications.

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**Table III. Schedule of procedures in TIME (day 0 is the day of revascularization)**

<table>
<thead>
<tr>
<th>Day 1 or 2 (consent)</th>
<th>Day 3/7 (SPI)</th>
<th>Day 4/8</th>
<th>Month 1</th>
<th>Month 3</th>
<th>Month 6</th>
<th>Month 12</th>
<th>Month 24</th>
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</thead>
<tbody>
<tr>
<td>Complete medical hx</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Incremental medical hx</td>
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<tr>
<td>Informed consent</td>
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<tr>
<td>Physical examination</td>
<td>X</td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Laboratory tests</td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<td>Pregnancy test*</td>
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<tr>
<td>ECHO</td>
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<tr>
<td>EKG</td>
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<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Bone marrow aspiration</td>
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<tr>
<td>Biorepository blood draws</td>
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<tr>
<td>Cardiac MRI</td>
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<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
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</tr>
<tr>
<td>Study product infusion (SPI)</td>
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<td></td>
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<td></td>
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<tr>
<td>Medication review</td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>AE/SAE evaluations</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Telemetry (18-24 h post-SPI)</td>
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<tr>
<td>Holter</td>
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<td></td>
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</tr>
</tbody>
</table>

SPI, Study product infusion; hx, history; AE/SAE, adverse event/serious adverse event.
*In women of childbearing age.
Patients with LVEFs <40% will be advised to take an aldosterone antagonist unless contraindicated by creatinine ≥2.5 or potassium ≥5.0. Follow-up schedules will follow a prespecified plan (Table III).

Safety monitoring
All CCTRN participants will be closely monitored for adverse events, and this information will be transmitted to institutional review boards of each center by the DCC; to the Food and Drug Administration (FDA), through the University of Texas Health Science Center–held investigational new drug (IND); and by the DSMB. The DSMB will meet at least twice yearly to review performance of the participating sites, to assess accruing safety data, and to ascertain feasibility of continuation of the study. Monthly safety and performance reports will be provided to the DSMB chair, the NHLBI Program Office, and the CCTRN Steering Committee Chair.

In addition, the DCC, under the direction of its Medical Director, will oversee and coordinate collection, standardization, integration, and analysis of study data from the various study components (enrolling sites and core facilities) and the preparation and distribution of the required reports to each of the safety oversight entities. The DCC will facilitate and monitor regulatory and safety compliance at each site and core laboratory and will conduct site visits to each site and core laboratory to assure protocol adherence and regulatory compliance, both on a regular basis and for cause.

Determination of outcomes
A 1.5-T cMRI scanner will be used, with precise magnetic resonance imaging protocols developed by the MRI core laboratory. Because resolution of myocardial stunning and improvement in global and regional LV function often continue to occur between day 3 and day 7, all patients will undergo baseline cMRI measurements at day 3. Commercial Siemens Argus analysis software will be used for measurement of global LV myocardial mass, volumes, and LVEF. Regional systolic wall motion, thickening, and radial displacement in the infarct and border zones will be determined. Areas of microvascular obstruction (MVO), infarct size, and degree of transmurality will be quantified by delayed, contrast (gadolinium)-enhanced MRI.

Wall motion imaging
Both global and segmental LV function will be obtained using a steady-state free-precession or fast gradient-echo technique. Long-axis cine images in the 2-chamber and 4-chamber projections will be obtained. In addition, a set of contiguous short-axis slices (8-10 mm thick) will be obtained from the mitral valve annulus through the apex of the LV throughout the cardiac cycle. Data will be analyzed using the Cardiovascular Angiography Analysis System/Magnetic Resonance Ventricular analysis software (PIE Medical Imaging BV, Maastricht, The Netherlands). Global parameters assessed will include end-diastolic volume, end-systolic volume, stroke volume, ejection fraction, and LV mass. Volumetric measurements will be performed by direct planimetry on the contiguous short-axis images at both end systole and end diastole. Regional measurements will include wall thickening and wall motion and will be calculated using 100 chords spaced every 3.6° originating from the centroid of the LV. Regional data will be reported using the AHA 17-segment model. The minimum spatial and temporal resolution requirements of the steady-state free-precession sequence are 2.5 × 2.5 millimeter voxels and 40 milliseconds, respectively.

Baseline perfusion imaging
A 2-chamber, long-axis cine image will be obtained based on axial scout images with the imaging plane spanning the center of the mitral valve coaptation point and through the apex of the LV. Based on this, a 4-chamber, long-axis cine image will be obtained. Subsequently, a T1-weighted gradient-echo baseline perfusion sequence will be performed using intravenous gadolinium (eg, gadolinium-DTPA). Three short-axis slices will be obtained (positioned from the 2-chamber and 4-chamber cine images) to encompass the basal, middle, and apical thirds of the LV during a bolus administration of gadolinium (0.15-0.2 mmol/kg). Imaging will be acquired for 60 dynamics per slice ensuring that the passage of contrast material through the myocardium is captured for semiquantitative analysis.

Viability imaging
Fifteen to twenty minutes after administration of gadolinium contrast agent, delayed-enhancement imaging will be performed with a T1-weighted inversion-recovery prepared gradient-echo sequence (DE-MRI). The inversion delay time will be iteratively adjusted for optimal nulling of normal myocardium. Contrast-enhanced viability imaging will be performed with 2 techniques: the standard 2-dimensional technique, which acquires a single slice each breath hold, will be performed in the short-axis projections using the same plane prescription as the functional short-axis cine series; and a high-resolution 3-dimensional technique will be used to acquire 10 short-axis slices during a single breath hold. Regions of irreversible myocardial damage are manifested by “hyperenhancement” (bright white areas) on the images, whereas normal and/or viable tissue is “nulled” (black) on the acquired images. The presence, location, and extent of irreversibly damaged tissue will be qualitatively and quantitatively assessed on a segmental basis. Pre- and posttherapy imaging, both cine wall motion and DE-MRI, will be
carefully matched for consistency and accuracy using internal landmarks including the insertion sites of the right ventricular freewall and the papillary muscle insertions.

**Statistical methods**

The TIME trial is a 2-factor experiment. The 2 factors are therapy (active vs placebo therapy) and timing (3 vs 7 days). The principal interest is whether the effect of cell administration timing influences the relationship between BMMNC infusion and cardiac function. Hypothesis testing for each of the primary end points will be carried out at the 0.05 level in this Phase II study. A total of 120 patients provide satisfactory power for the assessment of the overall effect of BMMNC administration compared to control for each of the 2 components of the primary end point (global and regional function) and permit an adequately powered inquiry into the influence of timing for each of the 2 coprimary end points. Assuming independence and normality of the observations, the sample size is calculated using the normal approximation to the 2-sample t test statistic. Final sample sizes were increased by 5% to compensate for patients who are lost to follow-up. The sample size of 120 patients is required to detect an absolute change of 5 absolute units in global ejection fraction and 7 absolute units in regional ejection fraction with 80% power at the 0.05 level. No correction for multiple comparisons will be made in this phase II study.

**Analyses**

The compatibility of baseline characteristics between the 2 treatment groups will be ascertained using standard normal tests (including t tests) for continuous variables and exact testing for categorical variables. The analysis variable will be the change in LVEF (one primary end point is global, the second is regional) from the immediate preinfusion level (day 3 MRI in patients randomized to day 3 therapy, and day 7 in patients randomized to day 7 therapy). Because each primary end point, global LVEF (%) and regional LV function, is a continuous variable, general linear mixed modeling will assess the effect of treatment on the primary end point of the study. In keeping with standard methodology for clinical trials, the primary analysis will compare the randomized study groups. Both unadjusted and adjusted treatment effects will be computed; adjustments will be for clinical site as well as for baseline covariates whose association with the dependent variable is generally accepted. Last observation carried forward procedures will be followed for patients who do not have a 6-month evaluation.

Both the effect of cell administration and the effect of the timing of cell administration will be evaluated for each of the secondary end points using procedures carried out for the primary evaluation. Logistic regression will be used to assess the effect of cell administration on the combined end point of death, reinfarction, repeat revascularization, and hospitalization for CHF.

The effect of subgroup stratum on the relationship between timing and timing’s influence on the cell delivery–end point (both primary and secondary end point) relationship will be assessed. If a treatment effect is demonstrated, it is not likely to behave identically among all important subgroups. The subgroups of interest are age, gender, race, hypertension history, diabetes mellitus, statin use, DES/BMS use, MVO, stent type (drug eluting stent versus bare metal stent), and LVEF (Table IV).

**Discussion**

Recent findings in animals that stem cell therapy significantly reduces the development of LV dysfunction after MI have been rapidly translated into clinical trials using a variety of cell types including intracoronary BMMNC administration. Importantly, these trials have demonstrated that cell delivery after AMI is safe over several years of follow-up, and meta-analyses review of these trials have found a small but significant improvement in LV function. However, despite these encouraging findings, many fundamental questions in cell therapy have not been addressed, including the important questions of optimal cell type, dose, and the timing of cell delivery post-AMI. The CCTRN was formed to address many of these unresolved issues, and this is the first clinical cell therapy trial sufficiently powered to focus on the important question of timing of cell delivery post-AMI.

### Table IV. Outcomes in TIME; active versus placebo comparisons

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Method of measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Coprimary</td>
<td></td>
</tr>
<tr>
<td>Δ Global LV function</td>
<td>cMRI</td>
</tr>
<tr>
<td>Δ Regional LV function</td>
<td>cMRI</td>
</tr>
<tr>
<td>II. Secondary</td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td>Clinical outcome</td>
</tr>
<tr>
<td>Reinfarction</td>
<td>Clinical outcome</td>
</tr>
<tr>
<td>Repeat revascularization</td>
<td>Clinical outcome</td>
</tr>
<tr>
<td>Hospitalization for HF</td>
<td>Clinical outcome</td>
</tr>
<tr>
<td>B. LV mass</td>
<td>cMRI</td>
</tr>
<tr>
<td>C. LVEDV</td>
<td>cMRI</td>
</tr>
<tr>
<td>D. LVESV</td>
<td>cMRI</td>
</tr>
<tr>
<td>E. Infarct size</td>
<td>cMRI</td>
</tr>
<tr>
<td>III. Additional/Subgroups</td>
<td></td>
</tr>
<tr>
<td>Age, gender, race, hypertension history, diabetes mellitus, statin use, DES/BMS use</td>
<td>Baseline characteristic</td>
</tr>
<tr>
<td>MVO</td>
<td>cMRI</td>
</tr>
</tbody>
</table>

HF, Heart Failure; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; DES/BMS, drug eluting stent/bare metal stent.
The timing of cell delivery after AMI is likely to be a critical factor in determining the efficacy of cell therapy due to the temporal changes that occur in the myocardium in the early days after AMI. These include the expression of growth factors and cytokines that may both promote cell survival and angiogenesis or, conversely, encourage myocyte apoptosis and adverse LV remodeling. Expression of chemokines, such as stromal-derived factor-1 that may aid in stem cell homing, are up-regulated in the infarct zone in the first few days after AMI. This is consistent with recent findings in patients demonstrating the homing of radiolabeled progenitor cells to the infarct zone being greatest in the first few days after AMI where their secretion of vascular endothelial growth factor and insulin growth factor-1 may improve perfusion and reduce apoptotic cell death in the infarct border zone. Conversely, a strong inflammatory reaction and release of reactive oxygen species in the infarct zone may adversely affect survival of the injected cells. In addition, there may be changes in the quality and quantity of harvested stem cells between the day 3 and 7 time point given the egress of cells from the bone marrow that occurs in this time window after AMI. These factors, among many, suggest that timing of stem cell administration post-AMI may be a critical component in dictating efficacy.

In the most published randomized clinical trials, BMMNCs were administered between 1 and 7 days post-AMI, but timing was not integrated into the randomization scheme. As a result, the designs of these trials were not sufficient to define the optimum timing of cell delivery after AMI. In a subgroup analysis, the REPAIR-AMI trial suggested that cell administration between 5 and 7 days was optimal in regard to recovery of LVEF. However, the ASTAMI trial administered BMMNCs in a similar time window and found no improvement in LVEF. However, this difference in outcomes may have also occurred due the different isolation procedure used in the ASTAMI trial (Lymphoprep) that may have resulted in reduced stem cell efficacy.

The number of infused cells administered to patients post-AMI has varied significantly between the randomized trials, with differences up to several orders of magnitude. Furthermore, the number of delivered cells within each trial has rarely been uniform, with some patients receiving up to 3 times the number of cells compared to other patients within the same trial. The failure to deliver a consistent cell dose remains a limitation of these trials. Only 1 published trial has attempted to address the issue of dose on its effects on LVEF. In that trial, 44 patients were randomized to 10 versus 100 million BMMNCs administered 5 to 9 days post-AMI where a slightly greater improvement in LVEF was observed in the high-dose cohort (5% vs 3%). A recent meta-analysis found no effect between the number of cells infused and recovery of LVEF. However, a second meta-analysis suggested that improvement in LVEF with BMMNC administration was dependent on the infusion of at least 100 million cells. Although the TIME trial is not a dose-ranging study, it will be the first major trial to administer the same number of cells to all of its patients (150 million), thus eliminating a potential variable that has not been controlled for in earlier trials.

The near exclusive use of autologous cells in cardiovascular cell therapy trials offers many important advantages from an immunologic and safety standpoint. However, recent research also suggests that the intrinsic efficacy of the cellular product may decline with age and important comorbidities, such as diabetes, that are common to the population being studied. The CCTRN is committed to the systematic exploration of cell phenotypes by the establishment of a Biorepository that will perform migration assays, measure nitric oxide and cytokine production, and characterize important receptor subtypes. In this randomized, phase II, double-blind, controlled study, a well-defined and translatable cell product and dose will be used in a relatively high-risk population. The timing of cell delivery will be addressed using time frames that are consistent with clinical applicability and an emerging safety profile. Additional human investigation in this and other clinical studies will provide a framework to complement ongoing basic science while further clarifying the therapeutic potential of cell delivery. Although this study is not designed to make head-to-head comparisons among cell types, it will generate a foundation for future studies to build upon within the CCTRN.

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